

Preparation of *E.coli* Culture Glycerol Stocks

This protocol is for the Preparation of *E.coli* Culture Glycerol Stocks

Transformation efficiency and subsequent analysis of recombinant plasmids depends on the quality of the *E.coli* strains used in laboratory. Proper storage is vital to ensure competent cells retain high transformation efficiency. Agar plates are only suitable for short term 4°C storage. A glycerol stock kept at -70°C is the ideal way to store bacterial strains.

1. When a lyophilized bacterial strain is purchased, a small portion of the powder is transferred with a sterile pipette tip or inoculating loop to liquid LB medium and incubated for 2-8 hours in a shaker.
2. A drop of liquid culture is spread on a selective plate and propagated overnight at 37°C to check for presence of selective markers.
3. Select a single colony and grow overnight.
4. Add 180 µl of 87% sterile glycerol to a 2 ml screw-cap culture vial.
5. Add 820 µl of liquid *E.coli* culture to vial, mix well, freeze in liquid nitrogen and store at -70°C.

When recovering bacteria from a glycerol stock, it is recommended to check for selective markers by streaking an aliquot on a selective plate.

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